

Optimization of Mobile Phase Composition for Preparative Separation of Profens Enantiomers by Chiral Liquid Chromatography

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Topic: Advancing the chemical engineering fundamentals.

Abstract

In this paper it will be presented and discussed the experimental and simulation results obtained for the preparative separation of ketoprofen and flurbiprofen enantiomers by chiral liquid chromatography. Experimental results include solubility studies, elution and frontal chromatographic experiments and the measurement of competitive adsorption isotherms, using different mobile phase compositions. Modeling and simulation tools are used to predict the behavior and the performance of fixed bed and simulated moving bed processes. These prediction tools are used to select the proper mobile phase composition for the enantiomers separation in a preparative and production-scale point of view.

1 Introduction

Ketoprofen (R,S)-2-(3-benzoylphenylpropionic acid) and Flurbiprofen (R,S)-2-(2-fluoro-4-biphenylpropionic acid) are both examples of profens, the actual most relevant subclass of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These drugs are frequently prescribed worldwide in relieve of pain and in the treatment of several forms of inflammation and in the treatment of main articular diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis (Burian and Geisslinger, 2005). The recent literature presents several studies concluding for different pharmacological proprieties for R and S profen enantiomers. Following these findings, chiral resolution of profen enantiomers can contribute to the development of new drugs with distinct therapeutic applications and/or safer pharmacological actions (Panico *et al.*, 2006).

The optimization of preparative chiral liquid chromatography and Simulated Moving Bed (SMB) processes is a complex task that requires a careful selection of mobile phase composition. In this selection, high resolution (or high selectivity) is not the only aspect, since other parameters, such as high solubility and low retention times, are crucial aspects that must also be considered (Ribeiro *et al.*, 2008).

In this work, experimental results obtained for the ketoprofen and flurbiprofen enantiomers will be shown, including solubility and adsorption measurements, and pulse and breakthrough experiments. Additionally, simulation results, based on the experimental adsorption isotherms measured, will be presented to compare the performance of fixed-bed and SMB processes.

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2 Experimental tools, modeling and simulation

The experimental tools, modeling and simulation procedures for the optimization of mobile phase composition are described in a recent published work (Ribeiro *et al.*, 2008). This includes experimental methods for solubility and adsorption isotherms measurements, and simulation tools to predict the operation of fixed-bed and simulated moving bed processes.

3 Results and Discussion

3.1 Solubility of profen racemic mixtures

Firstly, the solubility of ketoprofen and flurbiprofen enantiomers was measured in three pure solvents, all with a composition of 0.01%TFA: 100%n-hexane, 100%ethanol and 100%methanol. These solubility measurements were performed in duplicate and at a temperature of 23°C (room temperature). Obtained results (not shown) indicate that profens solubility increases when the solvent is changed from 100%n-hexane to 100% ethanol and from this to 100% methanol. On a second stage, the dependency of solubility with the alcoholic composition in an ethanol/n-hexane mixture was investigated. Results were consistent with the previous ones. Ketoprofen and flurbiprofen enantiomers have high solubility values in solvents with a high polar composition. On the other hand, a mobile phase with an increased polar content is referred as an advantage in preparative chromatography due, not only because it allows higher racemate solubility, but also because presents lower retention times (Lynam and Stringham, 2006).

3.2 Elution chromatography

Several experiments of elution chromatography (pulses) were performed on different solvent mixtures of ethanol/n-hexane and methanol/n-hexane, in order to characterize the system selectivity at preparative conditions. A preparative column (Chiralpak AD, Daicel, Japan), with a particle size of 20 μm , was used on these experiments. Six level concentrations were prepared in the range between 0.05 and 4.0 g/L, and injected using two different loops: 100 μL and 1 mL. Obtained results are presented in Figure 1, for ketoprofen, and in Figure 2, for flurbiprofen.

For ketoprofen enantiomers, results show that the 20%ethanol/80%n-hexane mobile phase presents considerable higher retention times than the pure mobile phases (ethanol and methanol). The hydrocarbon mobile phase also leads to important chromatographic tails, which is an indication of strong non-linear behavior and not welcome for preparative separations. Comparing the results obtained for the two pure alcohol mobile phases it can be clearly concluded that, despite higher ketoprofen solubility, pure methanol does not allows acceptable selectivity values and, consequently, ketoprofen enantioseparation.

For the flurbiprofen enantiomers, high selectivity values can be obtained for mobile phase compositions lower than 10%ethanol/90%h-hexane (results not shown). However, this mobile phase composition exhibits retention times substantially higher and very low solubility values, which means a clear disadvantage for high productivities. For an ethanol/n-hexane-based mobile phase, a 10/90 composition represents a reasonable compromise between selectivity, retention time and solubility. For methanol/n-hexane-based mobile phase, due to the immiscibility range between 6% and 60% methanol, its use is not possible. Taking into account the very low solubility values, experiments with less than 6% of methanol in n-hexane were also not carried out, since they are not attractive under preparative point of view. On the other side, and despite high solubility, the use of a mobile phase with higher methanol content (more than 60%) does not allow selectivity values as the ones obtained with ethanol/n-hexane mixtures.

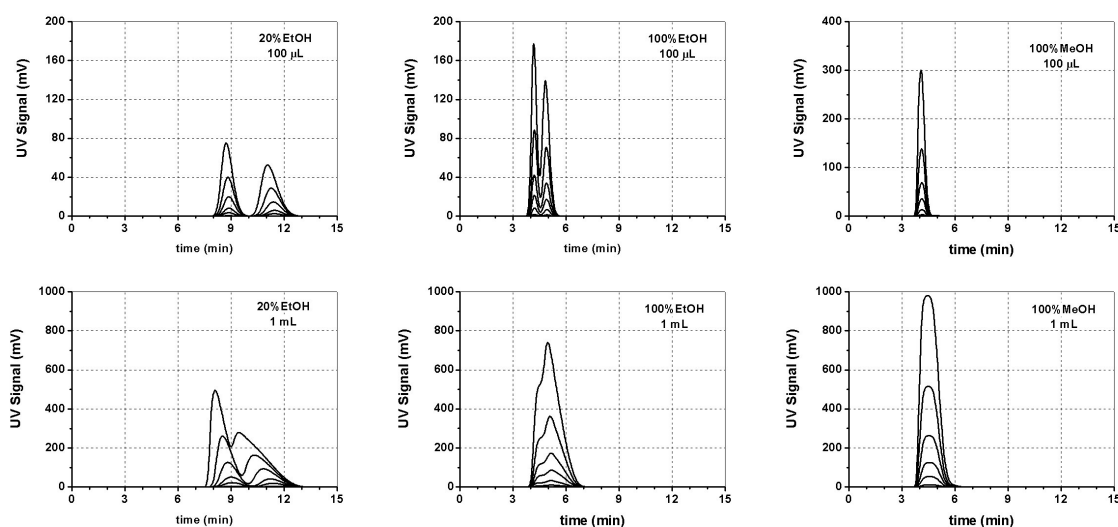


Figure 1. Experimental elution profiles of ketoprofen enantiomers in different mobile phase compositions: 20%ethanol/80%n-hexane, 100%ethanol and 100%methanol. Racemic ketoprofen concentrations in six different levels: 0.05, 0.2, 0.5, 1.0, 2.0 and 4.0 g/L; preparative column (particle diameter of 20 μ m); UV detection at 260 nm; flow rate of 1 mL/min; temperature of 23°C; injection volumes of 100 μ L and 1 mL.

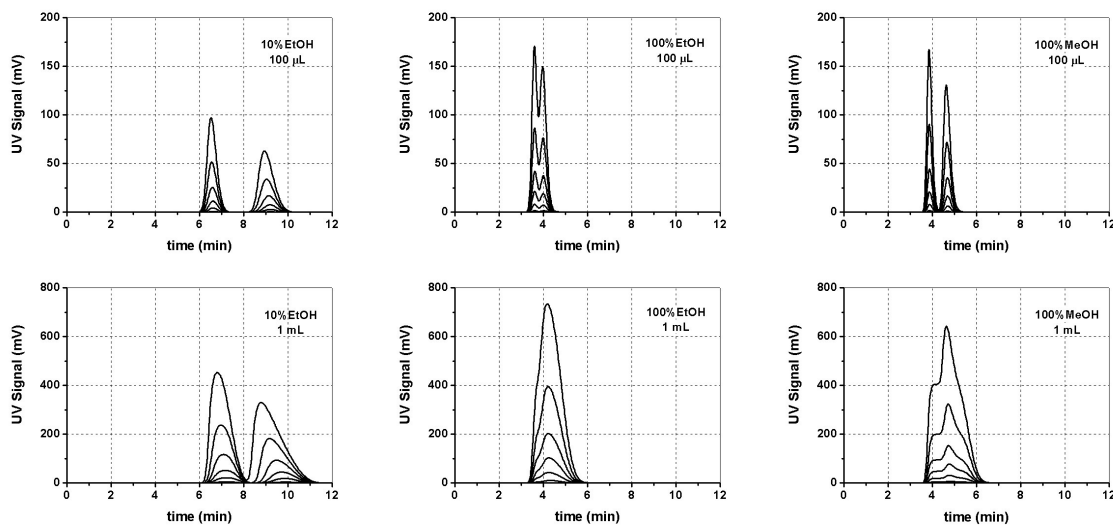


Figure 2. Experimental elution profiles of flurbiprofen enantiomers in different mobile phase compositions: 10%ethanol/90%n-hexane, 100%ethanol and 100%methanol. Racemic flurbiprofen concentrations in six different levels: 0.05, 0.2, 0.5, 1.0, 2.0 and 4.0 g/L; preparative column (particle diameter of 20 μ m); UV detection at 260 nm; flow rate of 1 mL/min; temperature of 23°C; injection volumes of 100 μ L and 1 mL.

3.3 Multicomponent adsorption isotherm experiments and modeling

Figure 3 (for ketoprofen) and Figure 4 (for flurbiprofen enantiomers) present the experimental results obtained for the adsorption isotherms measurements in different mobile phase compositions, showing a good agreement with model predictions. In these figures, the prediction of the selectivity factor and its concentration dependency is also represented.

Ketoprofen results clearly show three different situations: for 100%methanol, selectivity is low and constant, which means that the separation of ketoprofen enantiomers hardly can be achieved using pure methanol as mobile phase. Despite its high selectivity for low concentrations, the common 20%ethanol/80%n-hexane mobile phase presents a strong decrease in selectivity with the increase of enantiomers concentrations. The better situation

is obtained for 100%ethanol, where selectivity maintains high values even for high enantiomer concentrations.

Results obtained for flurbiprofen enantiomers confirm a decrease in selectivity with the increase of the alcoholic content. High selectivity values can be observed for high hydrocarbon content. However, this mobile phase composition exhibits retention times substantially higher and very low solubility values. As stated before, for the separation of the flurbiprofen enantiomers, a 10%ethanol/90%n-hexane mobile phase composition is a reasonable compromise between selectivity, retention time and solubility.

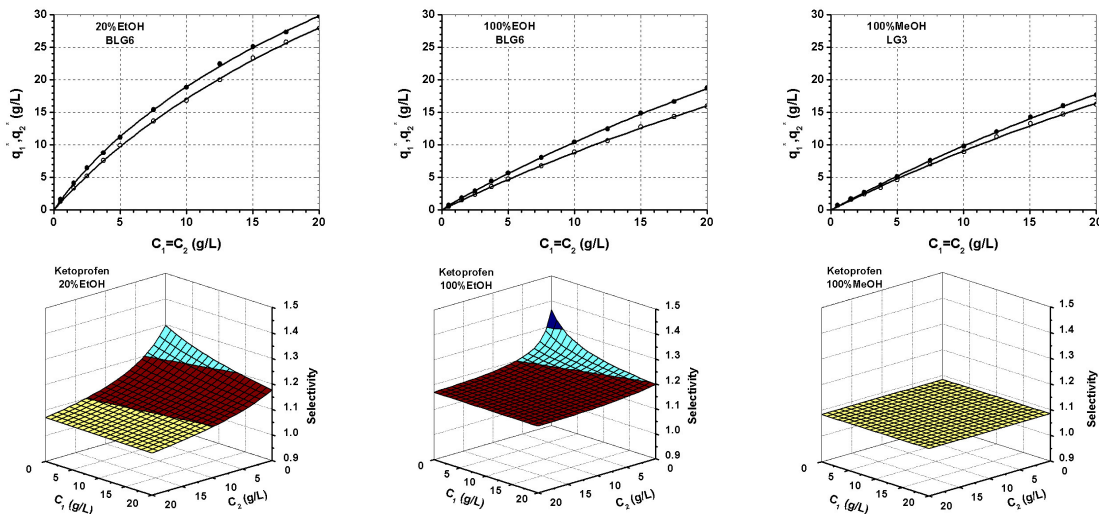


Figure 3. Comparison between model and experimental results for the equilibrium adsorption isotherms of ketoprofen enantiomers using the bi-Langmuir model (BLG6) in 20%ethanol/80%n-hexane and 100%ethanol and using the Langmuir model (LG3) in 100%methanol. Open and closed circles for the experimental concentration of the less and the more retained enantiomer, respectively; solid lines for the adsorption isotherm model. Temperature: 23°C. The second line presents the prediction of the selectivity factor as a function of both enantiomers concentration.

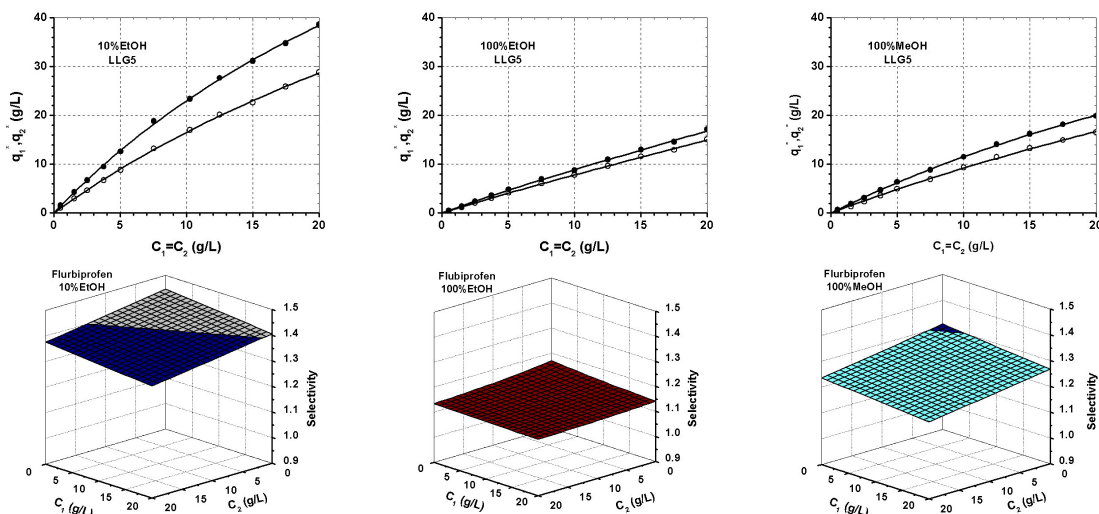


Figure 4. Comparison between model and experimental results for the equilibrium adsorption isotherms of flurbiprofen enantiomers using the modified linear+Langmuir model (LLG5) in 10%ethanol/90%n-hexane, 100%ethanol and 100%methanol. Open and closed circles for the experimental concentration of the less and the more retained enantiomer, respectively; solid lines for the adsorption isotherm model. Temperature: 23°C. The second line presents the prediction of the selectivity factor as a function of both enantiomers concentration.

3.4 Frontal chromatography experiments and simulation

Breakthroughs experiments were carried out with the purpose of testing the selected adsorption isotherm models. In the present work it is shown experiments using a racemic feed solution of 40 g/L and the selected mobile phase composition: 100%ethanol for the ketoprofen and 10%ethanol/90%n-hexane for flurbiprofen enantiomers. It can be clearly observed from Figure 5 that both the selected models describe very well the experimental data behaviour in the whole concentration range.

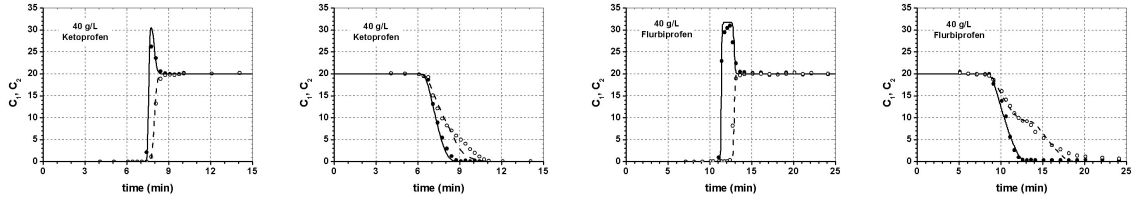


Figure 5. Saturation (adsorption) and regeneration (desorption) curves for a racemic feed concentration of 40 g/L. Comparison between experimental (points) and simulation (lines) results. Closed and open circles for the less and the more retained enantiomer, respectively. Temperature: 23°C. Flow rate: 0.5 mL/min. Ketoprofen: 100%ethanol and bi-Langmuir model (BLG6); Flurbiprofen: 10%ethanol/90%n-hexane and modified linear+Langmuir model (LLG5). Model parameters: $\varepsilon=0.4$, $Pe=3500$, $St=k\tau=1000$ (see Ribeiro *et al.*, 2008).

3.5 Performance of SMB operation

The performance of the ketoprofen enantiomers separation by SMB technology is compared in Figure 6 for different mobile phase compositions using the Equilibrium Theory model. The separation region (see plot $\gamma_3 \times \gamma_2$) for 20%ethanol/80%n-hexane has operating conditions considerable different from the ones obtained for the pure alcohol mobile phases (pure ethanol and pure methanol) due to the higher retention times. Comparing the separation regions for the three mobile phases, it can be concluded that, for the 20/80 composition, the separation region becomes quickly smaller with the increase of feed concentration. This is a sign of stronger non-linear behavior of the adsorption process and a reason for lower productivities.

The comparison of the SMB performance for the two pure alcohol mobile phases is clear: both have similar operating conditions due to similar retention times, but pure ethanol presents considerable better performances due to higher selectivity. Figure 6 also presents the productivity and the solvent consumption obtained for the different mobile phase compositions, as a function of feed concentration. These simulation results also clearly show that pure ethanol is the better choice for the separation of ketoprofen enantiomers through SMB operation: at high feed concentrations, the productivity using pure ethanol is three times and 25% of the one needed with the other two solvents, and solvent consumption is only 75% and 25% of the one needed with pure methanol and 20%ethanol/80%n-hexane, respectively.

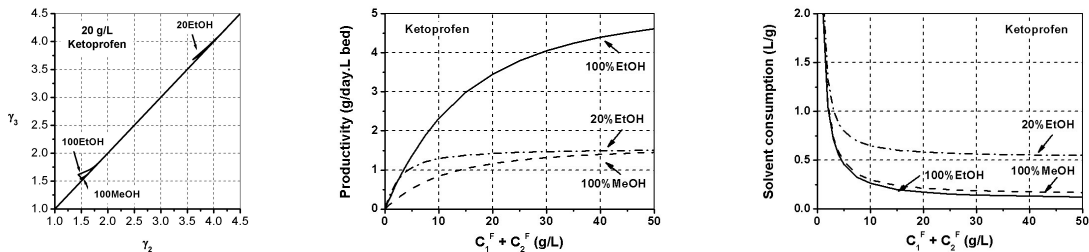


Figure 6. SMB separation regions and prediction of the performance of SMB operation for ketoprofen enantiomers: productivity and solvent consumption as a function of feed concentration for the different mobile phase compositions.

For flurbiprofen enantiomers (see Figure 7) it can be observed high retention time values in mobile phases with high hydrocarbon contents (the separation regions are progressively located at higher values of γ_2 and γ_3 with an increase of the n-hexane content). On the other side, these results are very different from the ones obtained for the separation of the ketoprofen enantiomers. The dimension of the separation regions progressively decrease with the decrease of the n-hexane content (increase of the ethanol content). Therefore, the best performance (bigger separation region) is obtained with a 10%ethanol/90%n-hexane composition through all feed concentration range. The performance parameters predictions also support the previous conclusions. Under preparative conditions, maximum productivity is achieved with the 10/90 composition, while solvent consumption does not significantly differ for all mobile phase compositions.

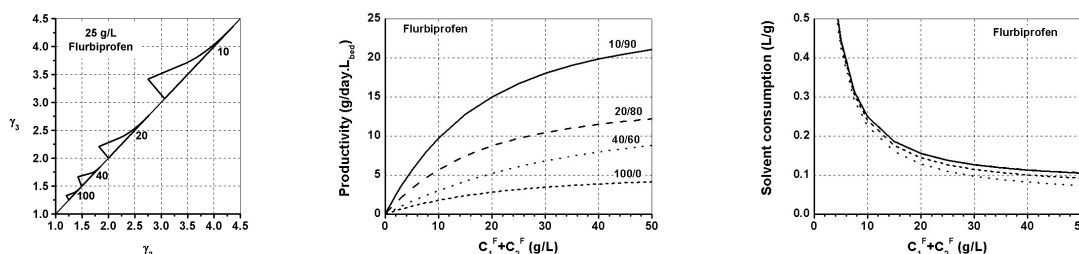


Figure 7. SMB separation regions and prediction of the performance of SMB operation for flurbiprofen enantiomers: productivity and solvent consumption as a function of feed concentration for the different mobile phase compositions.

4 Conclusions

For the separation of ketoprofen enantiomers, best situation is achieved using pure ethanol as mobile phase. For flurbiprofen enantiomers, the experimental results obtained lead to different conclusions: a 10%ethanol/90%n-hexane composition is the better choice since it represents a good compromise between selectivity, retention time and solubility. The present work shows that the choice of the proper mobile phase composition is a topic of utmost importance for the optimization of preparative liquid chromatographic separations. This choice will affect the throughput of the separation process since solubility, retention time, and selectivity are all parameters very sensitive to changes in mobile phase composition.

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